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The long-term objective of this proposal is to gain insight into mechanism of prostate cancer metastases to bone and attendant pathology of osteosclerosis. What is the cellular and molecular basis of osteotropism of metastatic prostate cancer in humans? The predominant site of prostate cancer is bone. However, unlike the osteolytic lesions of breast cancer, prostate cancer causes osteoblastic osteosclerosis which leads ultimately to morbidity and mortality. During the osteoscleorotic phase of prostate cancer metastases to bone there is increased bone formation. It is proposed to investigate the hypothesis that bone matrix is the "soil" that promotes the "seeds" of prostate carcinoma. Recent work has identified bone induction and stimulation by a family of bone morphogenetic proteins (BMPs). We have documented BMP 4 and BMP 7 expression in mouse prostate and BMP 6 in human prostate cells. It is plausible that metastatic human prostate cells produce BMPs which act in an autocrine-paracrine loop to stimulate bone formation and osteosclerosis based on their known motogenic, mitogenic and osteogenic actions. The specific aims of this hypothesis-driven proposal are (1) To investigate the expression of BMP antagonists and BMP receptors prostate cancer cell lines. (2) Generate clones of LNCaP cells with over-expression of BMP 6 and over-expression of BMP antagonist, DAN by stable plasmid transfections. Test the hypothesis DAN, a BMP antagonist binds to BMP 6. (3) Investigate the hypothesis that over-expression of BMP 6 leads to increased metastases to bone in a SCID mouse. The accrued knowledge of BMP signaling pathways including receptors and native antagonists, will help aid in the design of potential antagonists to block BMP signaling and reduce pain and pathologic fractures of bone in prostate cancer metastases.

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INTRODUCTION

Prostate Cancer is a horrendous affliction in men. In 2002 over 200,000 cases were diagnosed and nearly 39,000 patients died. Death is due to skeletal metastases, bone pain and bone fractures. A majority of prostate cancers metastasize to bone, especially pelvis and spine and causes pain and factors due to osteosclerosis. Bone morphogenetic proteins (BMPs) are signals to initiate new bone formation. Our research explores the BMPs, BMP antagonists and BMP receptors in Prostate Cancer. We set out to investigate BMP expression and BMP antagonist DAN in this Idea Development Award. The accrued knowledge will permit rational new approaches to treat bone pain and pathological fractures.

BODY

WORK ACCOMPLISHED

YEAR 1.

Task 1. Identify and characterize the expression of BMP 6 in human prostate cancer cells.

Task 2. Investigate and characterize expression of BMP antagonist DAN and BMP receptors

YEAR 2.

Task 3. BMP 6 Expression and Regulation

1. **BMP6 Is Highly Expressed In Human Prostate:** The human genome has at least 16 members of the BMP family, each with the ability to cause ectopic bone formation, and a growing number of BMP antagonists are being described. The goal of the studies presented here was to identify which BMP and BMP antagonist may most likely be involved in the pathology of prostate cancer metastatic foci. Real-time quantitative RT-PCR (reverse-transcription followed by polymerase chain reaction) using the TaqMan system was performed on total RNA (ribonucleic acids) isolated from human prostate biopsies obtained from the UCDMC (University of California at Davis Medical Center) urology department, and human prostate cancer cell lines. BMPs 2,3,4,6,7,8 were examined. The

results were normalized to the levels of the housekeeping GAPDH (glyceraldehyde-3-phosphate dehydrogenase) mRNA (messenger RNA). BMP6 was found to be highly expressed in prostate.

2. **Androgens Up-regulate BMP6 mRNA:** Our hypothesis was that BMP6 may affect its own expression in cultured human prostate cancer cells in an autocrine manner, and that this may be androgen dependent. To test this hypothesis, recombinant human BMP6 (rhBMP6) was added to the culture medium of LNCaP cells, and the BMP6 mRNA levels assayed by quantitative RT-PCR. Results were always normalized to the levels of GAPDH mRNA. Recombinant BMP6 added to hormone-free culture medium increased BMP6 mRNA levels 3 to 6 fold. In the presence of 100nM dihydrotestosterone (DHT), the same amount of recombinant BMP6 caused the LNCaP cells to increase BMP6 mRNA production 10- to 20-fold. The results of these experiments show that in cultured LNCaP cells, BMP6 can up-regulate its own expression, and that this up-regulation can be synergistically increased by the combined presence of BMP6 and the androgen dihydrotestosterone in the medium.

3. **BMP7 and Androgens Up-regulates BMP6 mRNA:** Previously work from our laboratory (Thomas *et al* 1998) has shown that BMP7 mRNA levels were significantly reduced in castrated versus intact mice, and that injections of androgens could restore the BMP7 mRNA levels in castrated mice. We tested the hypothesis that recombinant human BMP7 may also increase the level of BMP6 mRNA` when added to the media of cultured LNCaP cells. Real-time quantitative RT-PCR (using TaqMan chemistry) was performed on total RNA isolated from cultured LNCaP cells, and results from each culture condition were normalized to the levels of the housekeeping GAPDH mRNA. We found that added BMP7 increased BMP6 mRNA expression about 7-fold, and that in the presence of 100nM dihdrotestosterone, the same amount of added BMP7 caused a 20- to 30-fold synergistic increase in BMP6 mRNA production. An essentially identical experiment was performed with the osteosarcoma cell line SaOS-2. Unlike the LNCaP cells, the SaOS-2 osteosarcoma cells expression of BMP6 mRNA was not stimulated by androgens. However, BMP7 treatment increased the BMP6 mRNA expression as in LNCaP cells. This demonstrates the cell specificity of the androgen response.

4. **BMP-Antagonist DAN is Expressed in Prostate Cells:** The ability of BMP to bind to its receptors and initiate signal transduction pathways is regulated by BMP antagonists. These proteins bind to BMPs in the extracellular matrix thereby preventing BMP-receptor interactions. To identify which BMP antagonist may most likely be involved in prostate cancer, real-time quantitative RT-PCR

(using the TaqMan system) was performed on total RNA isolated from human prostate surgical specimens, and the LNCaP human prostate cancer cell line. BMP antagonists cerberus, chordin, DAN, gremlin and noggin were examined, and the results normalized to the levels of the housekeeping GAPDH mRNA. DAN was found to be expressed both in biposies and cancer cell lines. Furthermore, the addition of recombinant BMP6 to LNCaP cell culture decreased DAN mRNA expression. The other BMP antagonists tested did not show this inverse regulation by the addition of BMP6 protein to the cell culture medium.

5. **DAN Antibodies and Recombinant Protein:** The expression of DAN by prostate cancer cells, and its down-regulation by BMP6 in prostate cancer cells, are novel findings. Combined with the fact that DAN was originally identified as a tumor suppressor, this raises the intriguing possibility that an exogenous BMP antagonist, such as DAN, can be used to inhibit the bone-forming ability of BMP6. Perhaps this may lead to ways to lessen the severity of prostate cancer bony metastases. We have produced recombinant human DAN protein in a *Pichia Pastoris* yeast expression system (Invitrogen) in quantities sufficient for use in both *in vitro* BMP binding assays, and *in vivo* bioassays for BMP activity. To accomplish this, the cDNA coding for human DAN was cloned into the pPICZαA expression vector (Invitrogen), and subsequently introduced by homologous recombination into the genome of *Pichia Pastoris* at the methanol-oxidase locus. These yeast secreted substantial quantities of recombinant human DAN protein as a fusion protein with a myc epitope (useful for immunolocalization using commercially available anti-myc antibodies) and a 6-histidine tag (useful for purification of the protein due to its nickel binding properties). We have prepared two rabbit polyclonal antibodies raised against synthetic peptides, one corresponding to the amino-terminus of human DAN protein (peptide sequence KLALFPDKSAWCEAK), and one to the carboxy-terminus of human DAN protein (peptide sequence CGKEPSHEGLSVYVQGED). These antibodies cross-reacted with recombinant human DAN produced in yeast expression system. Thus, we have generated critical reagents for our work on BMP antagonists.

KEY RESEARCH ACCOMPLISHMENTS

- BMP 6 is highly expressed in human prostate cells
- Androgens increase BMP 6 messenger RNA
- BMP 7 increases BMP 6 messenger RNA

- BMP antagonist DAN is expressed in prostate cancer cells
- Antibodies to DAN was elicited and characterized. Recombinant DAN protein was expressed in yeast.

REPORTABLE OUTCOMES

1. Our research was presented at the UC Davis Cancer Research Conference
2. Dominik Haudenschild has received his Ph.D. degree.
3. Mr. Haudenschild was supported by the DoD grant during his training for Ph.D. degree.

CONCLUSIONS

The research demonstrates BMP 6 expression by prostate cancer cells. BMP 6 induces new bone formation as in osteosclerosis. Therefore, BMP antagonists such as DAN may ameliorate bone pain and fractures due to BMP 6 and may improve the quality of life for patients.

APPENDICES

Publications:

1. 2003 **Reddi, A.H.**, Roodman, D., Freeman, C., and Mohla, S. Mechanisms of Tumor Metastasis to the Bone: Challenges and Opportunities. J. Bone and Mineral Research, 18:190-194.

2. 2003 Moseley, T.A., Haudenschild, D.R., Rose, L., **Reddi, A.H.** Interleukin-17 Family and IL-17 Receptors. Cytokine & Growth Factor Reviews 14:155-174.

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Review

Mechanisms of Tumor Metastasis to the Bone: Challenges and Opportunities*

A HARI REDDI,[1] DAVID ROODMAN,[2] COLETTE FREEMAN,[3] and SURESH MOHLA[3]

ABSTRACT

In human cancers, bone is a common site for metastasis. It is well known that metastasis is the cause of morbidity and mortality in patients with cancer. Both breast and prostate carcinomas have a propensity to metastasize to bone. In general, metastatic breast cancers result in osteolytic lesions. On the other hand, prostate cancer metastases are osteoblastic and result in osteosclerosis. Thus, bone formation and bone resorption are at the crux of the cancer metastasis problem. For example, in the prostate, there is a vicious cycle of metastasis to bone (Fig. 1). Metastases to bone causes excruciating bone pain, pathological fractures, and eventually death, and therefore is a serious challenge to both bone biologists and cancer cell biologists. The stromal-epithelial interactions in breast and prostate are critical in initiation of carcinogenesis and the progression of the metastatic cascade to bone (Fig. 2). Over a hundred years ago, Stephen Paget enunciated the seed and soil hypothesis in which seeds of metastatic cancer cells of breast preferentially settle in the soil of bone matrix. Thus, the prostate/breast cancer bone interface and continuum has continuously presented challenges and opportunities and were discussed at a recent workshop. (J Bone Miner Res 2003;18:190–194)

Key words: prostate, breast, myeloma, bone morphogenetic proteins, parathyroid hormone

INTRODUCTION

THE TUMOR BIOLOGY and Metastasis Branch, Division of Cancer Biology of the National Cancer Institute, National Institutes of Health sponsored a workshop on "Mechanisms of Tumor Metastasis to the Bone: Challenges and Opportunities," in Bethesda, MD, November 29–December 1, 2000. The aim of the workshop was to encourage collaborations between bone cell biologists and clinical investigators and oncologists with expertise in cancer metastasis to the bone. Scientists from various disciplines of bone biology, including the bone microenvironment, extracellular matrix, and signal transduction, and from the field of tumor metastases, were invited. The goals of the workshop were to assess (1) the current state-of-the-science on the available experimental models to study bone microenvironment in metastasis. (2) What is currently known about molecular mechanisms of tumor-bone stroma interaction? (3) What are some of the critical unresolved issues in tumor metastasis to the bone that should be emphasized by new investigations? The scientific interactions were intended to foster progress in reducing the morbidity and ultimate mortality that result from tumor metastases to bone. This article presents the background and highlights of the meeting to a wider audience of bone biologists and it is anticipated it might provide an impetus for further research on tumor metastases to bone.

*Participants: Regis Bataille, Tatiana Byzova, Michael Cher, John Chirgwin, Leland Chung, Denis Clohisy, William Dalton, Caroline Damsky, Rick Derynck, Patricia Ducy, Joshua Epstein, Colette Freeman, Michael Freeman, Carol Gay, Robert Getzenberg, Joan Goldberg, David Goltzman, Jeffrey Green, Theresa Guise, Hynda Kleinman, Beatrice Kundsen, Paul Kostenuik, Allan Lipton, Andrea Mastro, Suresh Mohla, Kenneth Pienta, Hari Reddi, Pamela Gehron Robey, David Roodman, Thomas Rosol, Neeraja Sathyamoorthy, Dinah Singer, Frederick Singer, Gary Stein, Gordan Strewler, Steven Teitelbaum, Erik Thompson, Gabri van der Pluijm, Matt Van Eman, Charlene Waldman, Katherine Weilbaecher, Toshiyuki Yoneda, Bruce Zetter, and Haiyen Zhau.

The authors have no conflict of interest.

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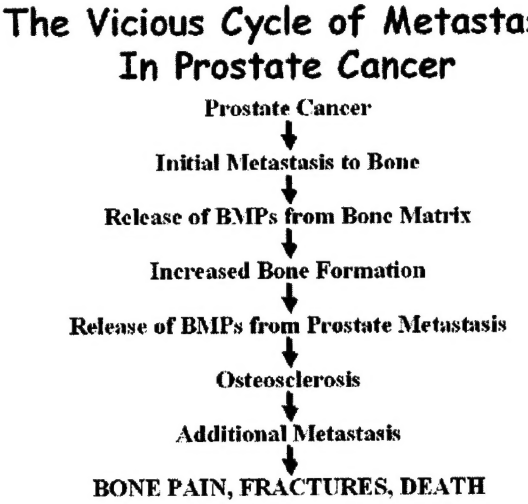

FIG. 1. The vicious cycle of metastasis cascade in prostate cancer, with special reference to increased bone formation and osteosclerosis. It is well known that BMPs are the primordial signals for new bone formation.

MODELS OF METASTASIS

The interdisciplinary nature of the workshop necessitated overview talks on bone biology, development and morphogenesis, bone metastasis, and experimental models of bone metastasis by Roodman, Goltzman, and Yoneda, respectively.[1–5]

The second session, devoted to models of bone metastasis, included breast cancer, prostate cancer, and myeloma. Guise and Greene presented experimental models to study breast cancer.[6] Chung and Cher presented models to study prostate cancer bone metastasis.[7–9] Epstein, Bataille, and Dalton discussed models of multiple myeloma.[10–12] Key issues covered by these speakers included the role of angiogenesis, molecular mechanisms of bone metastasis, and the role of bone stroma.

The next session focused on understanding bone metastasis. Key issues discussed in this session included mechanisms of osteoclastic bone metastasis, discussed by Teitelbaum and Kostenuik,[15,16] and the mechanisms of osteoblastic metastasis, discussed by Strewler and Clohisy.[15–17] Speakers focused on the role of tumor-derived factors that stimulate osteoblastic and/or osteoclastic activity, the dynamic and reciprocal interactions between tumor cells and bone, and the potential for using the resulting information for therapeutics. It was also realized that the progressive bone resorption and formation occurring with tumors can be monitored by bone markers and can be ameliorated with drugs including bisphosphonates.

THE BONE MICROENVIRONMENT AND EXTRACELLULAR MATRIX

Damsky[17] presented a detailed discussion on adhesion molecules and cell signaling, in particular, those associated with tumor cells and matrix interactions. Integrin signaling affects bone cell function and osteoclast recruitment, and Damsky raised the interesting question, "does adhesion molecule mimicry play a role in tumor cell homing?" She suggested that tumor invasion might be similar to trophoblast invasion in pregnancy, which is also dependent on angiogenesis and adhesive cell interactions. She emphasized that the trophoblast may be an appropriate model for studying the mechanism of tumor metastasis. Robey[18] discussed the composition of the bone marrow microenvironment and suggested that it is really a colloidal suspension with little extracellular matrix. She noted that there were many differences between the in vivo and in vitro microenvironment. She raised the important issue regarding the cell types in the bone microenvironment that are associated with tumor cells, and provided data showing that cancers with a propensity to metastasize to bone express bone sialoprotein and osteopontin. She suggested that bone sialoprotein production by tumor cells may "shield" the tumors from the immune system.

From the pioneering work of Huggins, it is well known that prostate cancers metastasize to bone and result in osteoblastic and osteoclerotic lesions. Implantation of mineral-free extracellular matrix of bone[19] in ectopic sites resulted in new bone formation. A family of bone morphogenetic proteins (BMPs) have been identified, isolated, and cloned[20] from bone matrix. Reddi[21] discussed the potential role of prostate-derived BMPs in osteosclerosis during prostate cancer metastases to bone.

Kleinman[21] explored the question as to why breast and prostate cancer metastasize to bone and suggested that specific homing factors in bone facilitate growth of cancer in bone. She provided data that osteonectin may be responsible for chemotaxis of tumor cells to bone. Freeman[22] suggested that the EGF receptor might be important in mediating tumor cell progression in prostate cancer. He presented data to show that the transcription factor BAG-1 may have an important role in prostate cancer metastasis. Derynck[23] provided evidence that TGF-β has a dual effect in bone when produced by tumor cells, by increasing both osteoblast proliferation and osteoclast activity, while decreasing the expression of bone-specific transcription factor Cbfa1. Zetter[24] discussed the observation that the prostate is the only organ that secretes polyamines. The polyamine, spermine, inhibits the growth of prostate cancer cells. Ornithine decarboxylase (ODC) is a key enzyme in polyamine biosynthesis, and ODC degradation is mediated by association with antizyme. High polyamine levels induce antizyme in spermine sensitive cells but not in spermine-resistant prostate cancer cells. These data suggest that induction of ODC antizyme may be a novel therapeutic approach in prostate cancer. Overall, the session explored the current understanding of how the bone microenvironment enhanced tumor cell growth, the important role of bone-tumor interaction in producing bone metastasis, and why bone may act as a sanctuary for tumors. The results suggested several potential therapeutic approaches and targets.

MECHANISMS OF OSTEOCLASTIC AND OSTEOBLASTIC METASTASES

Teitelbaum[13] reviewed the physiology of osteoclast function and the contribution of bone lysis to metastasis. He

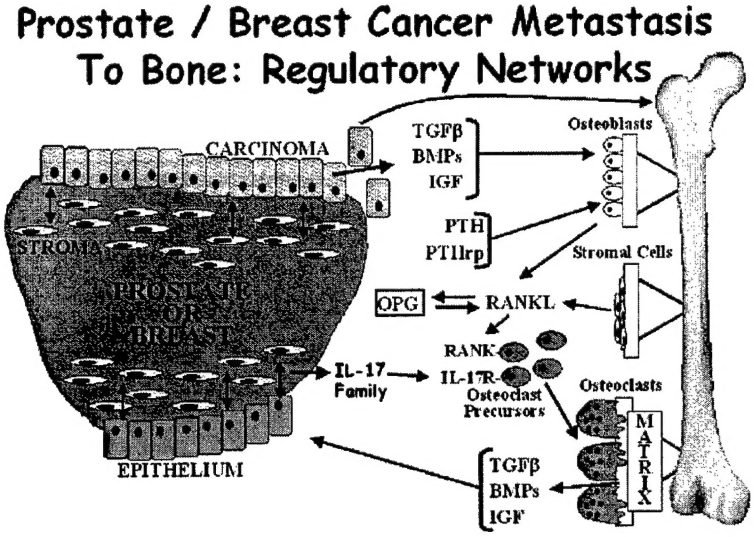

FIG. 2. The breast/prostate cancer metastasis to bone. In general, metastatic breast cancers are osteolytic and prostate cancers are osteosclerotic. The dynamic regulatory networks at the interface of breast/prostate carcinoma metastases and bone are indicated in a simplified form. The interactions between stromal cells and epithelial cells are critical for tumor progression and metastasis in both breast and prostate. The carcinoma cells secrete morphogens and growth factors such as BMPs, IGF, and TGFβ, which act on cells in bone. In response to PTH and PTHrp, osteoblasts and stromal cells secrete RANKL, which binds to RANK on osteoclast precursors to differentiate into functional multinucleate osteoclasts. The bioavailability of RANKL to its receptor RANK is determined by the activity and affinity of a soluble decoy receptor osteoprotegerin (OPG) to RANKL. Interleukins 1 and 17, TNF, and the cognate signaling system have a role in osteoclastogenesis. The degradation of bone matrix by multinucleate osteoclasts releases growth factors and morphogens from the extracellular matrix such as BMPs, IGF, and TGFβ family members. The regulatory networks in the breast/prostate cancer metastasis to bone are reciprocal and dynamic as illustrated by the secretion of BMPs, IGF, and TGFβ family of ligands, cognate receptors and antagonists, and binding proteins for the growth factors and morphogens. The binding proteins include IGF BPs, latent TGFβ binding proteins, and BMP antagonists—Gremlin, Cereberus, and DAN.

stressed that understanding the function of these cells would allow for development of new drugs directed at specific targets in the cell, which would inhibit osteoclast activity in clinical diseases such as bone metastases. A demonstration of the success of this approach was described in a mouse model of osteoporosis. Because of the observation that integrin $\beta 3$ knockout mice produce osteoclasts with impaired ability to resorb bone, a RGD mimetic agent was synthesized and tested in ovariectomized mice. Administration of the drug-inhibited bone loss usually seen with the loss of estrogen.

Kostenuik[14] reviewed osteoclast function with emphasis on osteoprotegerin and RANK as the dominant influence on osteoclast function (Fig. 2). He discussed the evidence that bone resorption induced by bone metastases could increase skeletal tumor burden by release of growth factors from the bone matrix. It was emphasized that histology of the skeleton is critical in assessing the efficacy of drug treatment than skeletal radiographs. He presented data that osteoprotegerin (OPG) could decrease skeletal tumor burden in a colon cancer animal model, although the underlying mechanisms remains to be defined. A final issue, which was raised, was the possibility that tumor cells might directly degrade bone, and if this is significant, osteoclast suppression alone might not be maximally effective.

Strewler presented his unpublished data on the development of a mouse model of prostate cancer bone metastases. He modified a clone of the parental cell line, LNCaP, by overexpressing Her2/neu and prostate specific antigen (PSA). After direct injection of cells into mouse femora, an excellent osteoblastic metastasis response was produced. The skeletal lesions were evaluated by three-dimensional computed tomography (CT) scan and histomorphometry. He is currently using a mouse calvarial bone formation assay to purify and identify a soluble mediator of bone formation produced by the tumor cells. Clohisy[15,16] discussed bone metastases from a clinical viewpoint, indicating three stages of progression: tumor growth, bone pain, and fracture. Many prostate cancer patients have bone marrow micrometastases at the time of initial diagnosis. These may progress to an asymptomatic stage in which the bone scan is positive. Finally, further growth is associated with pain and fractures. He stressed that the goal should be to kill the tumor in the microscopic state and proposed designing studies directed toward determining how bone marrow cells could be used to kill micrometastases.

BONE MARKERS AND STRATEGIES FOR TARGETING BONE

The last session was dedicated to approaches that can be used to target the bone cells once metastasis has already occurred. The presentations emphasized that the knowledge acquired by bone biologists in understanding both the genetic program specific to bone cells and the pathways that can be targeted to treat metabolic bone diseases such as osteoporosis can also be used in the particular setting of treating bone metastases. Ducy[25] presented a general overview of the genetic opportunities available to target bone cells using cell-specific promoters. Particular emphasis was

given to the possibility of using osteoblast-specific promoters such as osteocalcin[26] or osteoclast-specific markers (e.g., tartrate resistant alkaline phosphatase) to overexpress endogenous factors or express exogenous factors in the bone microenvironment. Such factors might decrease the bone cell response to signals produced by metastatic cells or induce apoptosis of the cancer cells. Stein[27] presented the results that his laboratory obtained in specific targeting of osteoblastic cells using a transgenic construct containing the rat osteocalcin promoter driving the CAT reporter gene. He demonstrated that bone marrow stem cells transfected ex vivo with such constructs show expression in bone once they are reintroduced into the animal. He also presented his findings regarding the interaction between cell-specific transcription factors and chromatin structure. This part of his presentation emphasized the possibility of manipulating gene expression by targeting another level of genetic regulation. Chung[28] showed that once localized in the bone microenvironment, prostate cancer cells undergo a modification of their genetic program toward an osteoblastic phenotype. He showed that these cells progressively express typical markers of osteoblasts, including osteocalcin. This genetic "transdifferentiation" of prostate cancer cells was used to target the metastatic cells by a genetic approach. Tumor metastasis was inhibited in animals treated with an adenoviral-osteocalcin promoter-thymidine kinase suicide construct.

The final presentations by van der Pluijm and David Roodman focused on the biology and use of bisphosphonates.[29,30] This class of molecules was developed to treat osteoporosis, specifically targeting bone by their ability to be integrated in the mineralized bone matrix and to act locally on osteoclasts to block bone resorption. Recent work has demonstrated that bisphosphonates also have a direct effect on cancer cells by inhibiting their attachment to the bone matrix, inhibiting the actions of the matrix resorbing metalloproteinases, and promoting apoptosis of the tumor cells. The efficacy of treating patients with bone metastasis by bisphosphonates was also demonstrated.

KEY ISSUES AND RESEARCH OPPORTUNITIES

A wide-ranging general discussion among the participants at the workshops raised additional new questions. What are the unique features of bone that encourage metastases of tumors? What are the benefits and limitations of our current models? What is the role of angiogenesis in experimental models? What are the characteristics of breast and prostate cancers that promote metastasis to bone? Why does myeloma lead to bone destruction? Are there "homing" sites in bone microenvironment? Do chemokines play a role in homing? What is the role of bone in providing a "chemoresistant sanctuary" to tumor cells?

The following issues were raised during the discussion. It is unlikely that a single experimental model will mimic the whole process of in vivo tumor metastases. Thus, each animal model is useful to examine certain key steps or phases of the metastatic cascade. Also, while there may be differences among metastatic breast, prostate, and myeloma cells, the similarities may have critical translational implications for treatment and prevention of cancer-induced skeletal fractures. The interface between bone cell biology and tumor epithelial-stromal interactions including extracellular matrix is critical for understanding bone metastases. Development of transgenic animal models with tissue and cell-specific promoters to overexpress dominant negative mutant receptors may shed new insights into the metastatic process. Although cell cultures are of use in studies on metastasis, the lack of three-dimensional tissue organization afforded by aggregate cultures or organotypic models has to be borne in mind.

Other challenges and issues, which remain elusive, are as follows. Do metastatic tumor cells need the extracellular matrix of bone for adhesion and survival? When and how do metastatic tumor cells become autonomous? Is there cell and tissue mimicry by tumor cells to imitate the bone microenvironment? What is the role of fibronectin and its integrin receptors in both normal and metastatic bone formation?

Finally, some topics on the subject of metastatic cancer to bone may be topics for a follow-up workshops in future. For example, (1) the role of angiogenesis and vascular invasion in tumor-bone metastases; (2) the entire issue of bone pain: causes and therapeutic approaches, and the role of cyclooxygenase 2 inhibitors; (3) role of matrix metalloproteinases and their cognate inhibitors such as tissue inhibitors of metalloproteinases in tumor metastases to bone; (4) why is bone a specific sanctuary for prostate and breast cancers; (5) are there bone-derived growth factors, cytokines, chemokines, and BMPs, which locally modify and amplify metastatic microfoci to metastases and cause pathological bone fractures; (6) do bone matrix components and BMPs confer selective advantage to tumor cells in terms of chemotaxis, mitosis, and differentiation: what is the role of stromal and immunomodulatory cells; (7) do the sinusoidal endothelial cells play a role in the micro-metastatic foci: how do vascular endothelial growth factors and their cognate receptors regulate initial seeding and metastatic growth?

RECOMMENDATIONS AND CONCLUSIONS

The key areas recommended for further investigation include the following: molecular cell biology of transcriptional and translational control of bone formation and resorption in metastatic foci; molecular markers of bone formation and resorption; genomics and proteomics of the sequential cascade of metastasis to bone; influence of stromal-epithelial interactions on growth, differentiation and metastases of cancers to bone; signaling systems during metastasis to bone; dynamic regulatory networks of cytokines, chemokines, growth factors, and morphogenetic proteins and cognate receptors in the interface of metastatic tumors and bone; novel animal models based on transgenic mice and conditional knockouts; real-time imaging of tumor metastatic cascade and interaction with compartments of bone; and use of the advances in molecular cell biology and imaging of metastases to bone in molecular therapeutics targeted to disruption of the metastatic code.

In conclusion, this timely workshop was an exciting forum for insights into the intricacies of tumor metastasis to bone. Cancer research based on genetics, genomics, mi-

croarrays, and proteomics has made giant strides. However, the tumor metastases to bone and the interface has lagged and requires renewed scientific vigor and therefore additional support. The recent advances in molecular cell biology of bone formation resorption and remodeling augers well for the future investigations into tumor metastases to bone.

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Survey

Interleukin-17 family and IL-17 receptors

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Abstract

Interleukin-17 (IL-17) is a pro-inflammatory cytokine secreted by activated T-cells. Recently discovered related molecules are forming a family of cytokines, the IL-17 family. The prototype member of the family has been designated IL-17A. Due to recent advances in the human genome sequencing and proteomics five additional members have been identified and cloned: IL-17B, IL-17C, IL-17D, IL-17E and IL-17F. The cognate receptors for the IL-17 family identified thus far are: IL-17R, IL-17RH1, IL-17RL (receptor like), IL-17RD and IL-17RE. However, the ligand specificities of many of these receptors have not been established. The IL-17 signaling system is operative in disparate tissues such as articular cartilage, bone, meniscus, brain, hematopoietic tissue, kidney, lung, skin and intestine. Thus, the evolving IL-17 family of ligands and receptors may play an important role in the homeostasis of tissues in health and disease beyond the immune system. This survey reviews the biological actions of IL-17 signaling in cancers, musculoskeletal tissues, the immune system and other tissues.

Keywords: Interleukin-17; T-cells; Receptors; Prostate; Cartilage; Cancer

Contents

1. Introduction

Interleukin-17 (IL-17A) is a cytokine secreted exclusively by activated T-cells. IL-17 cDNA has been isolated and cloned from the murine hybridomas (cytotoxic T lymphocyte antigen 8 (CTLA-8)) [1,2] and has homology to open reading frame 13 from the T lymphotropic Herpesvirus saimiri. The human IL-17A gene product is a protein of 150 amino acids with a molecular weight of 15 kDa, and is secreted as a disulfide linked homodimer of 30–35 kDa glycoprotein [3].

Five related cytokines were identified, through database searches and degenerative RT-PCR, that share 20–50% homology to IL-17. IL-17 has been designated IL-17A to indicate that it is the founding member of the IL-17 cytokine family. The shared features of the IL-17 cytokine family include conserved cysteines which, in IL-17F [4], have been shown to exhibit the features of a classic cystine knot structural motif found in bone morphogenetic proteins

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(BMPs), transforming growth factor beta (TGF-β), nerve growth factor (NGF) and platelet-derived growth factor BB (PDGF-BB) [5]. IL-17F, like IL-17A, is produced primarily in activated T-cells. In contrast, IL-17B, IL-17C, IL-17D, and IL-17E are expressed in a wide assortment of tissues. Their functions partially overlap those of IL-17A, although they have not been as thoroughly investigated.

The receptor for IL-17A (IL-17R) is a single-pass transmembrane protein of approximately 130 kDa. While the IL-17A cytokine is expressed only by T-cells, its receptor is expressed in all tissues examined to date. The activation of the receptor by IL-17A generally results in the induction of other pro-inflammatory cytokines, through the activation of NF-κB.

Four additional receptors have been identified, through database searches, which share partial sequence homology to IL-17R. Of these, only IL-17RH1 (also called IL-17B receptor) has been shown to bind to IL-17 cytokines, namely IL-17B and IL-17E [7,36]. IL-17 receptor-like protein (also called IL-17RL or IL-17RC), IL-17RD (also called SEF or IL-17RLM) and IL-17RE have only been identified by sequence similarity to IL-17R. Many of these receptors exist as alternatively spliced isoforms, some of which may not contain transmembrane or cytoplasmic domains, and thereby may be acting as soluble decoy receptors. They exhibit a broad tissue distribution, and not much is known about their functions or signal transduction pathways.

With the newly identified family of IL-17 cytokines and receptors, and their expression in disparate tissues, the scope of IL-17 cytokine activity and expression extends beyond the T-cell immune system mediated inflammatory response. IL-17 cytokines and their receptors thus may play an important role in the homeostasis of tissues and the progression of disease.

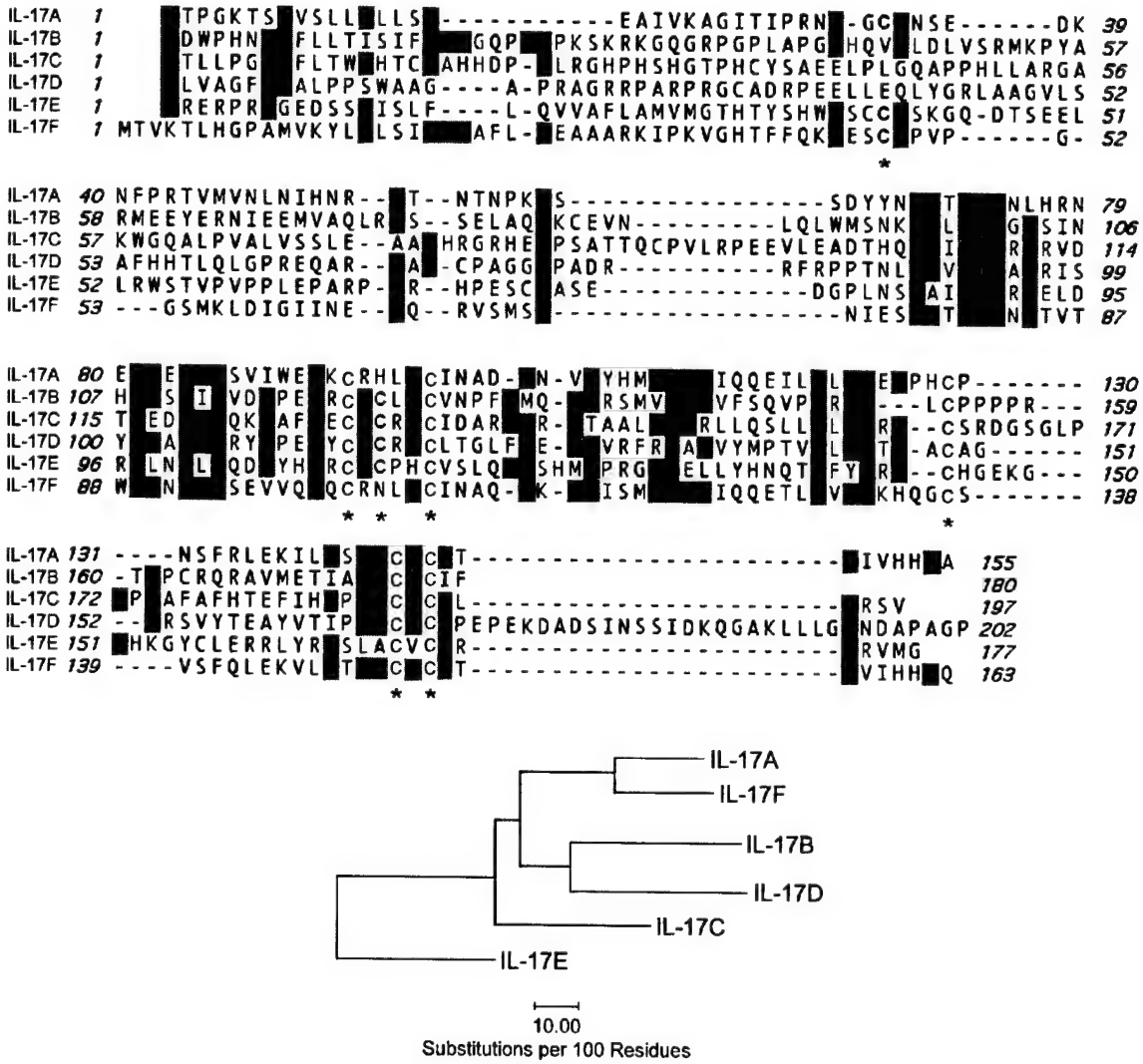

Fig. 1. IL-17 cytokine family alignment: alignment of human IL-17 cytokine family members shows their common features. Darker shading and boldfaced type represent sequence identity. The conserved cysteins are in red which may be involved in intra- and inter-chain disulfide bonds. The dendrogram shows how these cytokines are evolutionarily related.

2. IL-17 family overview

Proteins with significant homology to IL-17 have been identified recently with the continuing advances and accumulating information in expressed sequence tags (ESTs), genomics and proteomics databases. Some of these cytokines have alternative names as they were originally identified in other systems. These related proteins have been grouped and designated IL-17A–F. Fig. 1 shows an alignment of human IL-17 cytokines, with identical residues darkly shaded and boldface. There are five highly conserved cysteines highlighted in red, four of which have been shown to form a cystine knot in the crystal structure of IL-17F [6]. This cystine knot is similar to a common structural motif found in growth factors such as BMPs, TGF-βs, NGF and PDGF-BB, except that in these other growth factors the cystine knot is formed with six cysteines rather than four. Similar to many growth factors, members of IL-17 family of ligands are expressed as tightly associated dimers (IL-17B) [7] or disulfide-bonded homodimers (IL-17F) [6].

The dendrogram shown in Fig. 1 depicts the interrelationships and the degree of similarity amongst the members of the IL-17 cytokine family. IL-17A and IL-17F share the highest degree of homology, being 50% identical to each other. It is interesting to note that these also map to the same chromosomal location, 6p12. IL-17B through E are less related, sharing only 16–30% identity at the primary sequence level, and they each map to a different chromosome. The accession numbers, chromosomal locations in the human genome, and alternative names are presented in Table 1. These cytokines are well conserved in the mouse, with 62–88% similarity between the human and mouse homologs.

Proteins with significant homology to the IL-17 receptor have been identified using sequence similarity searches of genome databases. These proteins share only limited similarity with each other, and do not contain conserved domains present in other proteins. All are single-pass transmembrane proteins with an extracellular amino-terminus. The accession numbers, chromosomal locations in the human genome, and alternative names are presented in Table 1. These receptors are well conserved in the mouse, with 68–90% similarity at the protein level between the human and mouse homologs. IL-17RH1 and IL-17RD are both mapped to the same chromosomal location, 3p21.1, as are IL-17RL and IL-17RE which both map to 3p25.3.

The genomic structure of the IL-17 receptor family of proteins is shown in Fig. 2. All receptors are transcribed from multiple exons, ranging from 11 in IL-17RH1 to 19 in IL-17RL. With the notable exception of IL-17R, there is extensive evidence of alternative splicing of these receptors, which is diagrammed by lines connecting adjacent exons in splice variants in Fig. 2. The alternative splicing of IL-17RH1 and IL-17RL has been shown to create frame-shifts and introduce stop codons which result in secreted soluble proteins [8,9]. These soluble proteins presumably retain their ligand-binding properties, yet lack signal transduction capability thereby acting as soluble decoy receptors. There is also evidence of alternative splicing of IL-17RE in the EST database, although the effects on the protein have not been documented. Alternative transcription start sites are evident in the various isoforms of IL-17RD, which produce proteins named IL-17RLM long and IL-17RLM short, and there are reports of an alternative translational start site in this gene which produce a protein named SEF [10,11].

3. IL-17 family in cartilage and arthritis

To provide a suitable context for understanding the actions of IL-17 cytokines in cartilage and arthritis, we provide a brief overview of cartilage function and tissue homeostasis.

Table 1
Identification of IL-17 family

Name	Alternate name 1	Alternate name 2	Chromosome location	Human protein accession number	Human mRNA accession number	Mouse protein accession number	Mouse mRNA accession number	Homolgy to human (%)
Ligands								
IL-17A	CTLA-8		6p12	NP_034682	NM_010552	NP_034682	NM_010552	62
IL-17B	CX1	NERF	5q32	NP_055258	NM_014443	NP_062381	NM_019508	88
IL-17C	CX2		16q24	NP_037410	NM_013278	NP_665833	NM_145834	83
IL-17D	IL-27	IL-27A	13q11	NP_612141	NM_138284	NP_665836	NM_145837	78
IL-17E	IL-25		14q11.1	NP_073626	NM_022789	NP_542767	NM_080729	81
IL-17F	ML-1		6p12	NP_443104	NM_052872	NP_665855	NM_145856	77
Receptors								
IL-17R	IL-17AR		22q11.1	NP_055154	NM_014339	NP_032385	NM_008359	68
IL-17RH1	IL-17BR	Evi27	3p21.1	Q9NRM6	NM_014339	Q9JIP3	NM_019583	82
IL-17RL	IL-17RC		3p25.3	NP_116121	NM_032732	NP_598920	NM_134159	71
IL-17RD	SEF	IL-17RLM	3p21.1	AAM77571	AF458067	NP_602319	NM_134437	90
IL-17RE			3p25.3	NP_653241	NM_144640	NP_665825	NM_145826	82

A list of known IL-17 family ligands and receptors with their alternate names. The National Center for Biotechnology Information (NCBI) accession numbers for human protein and mRNA as well as their mouse counterpart. The percent homology is based upon human and mouse protein sequence similarity.

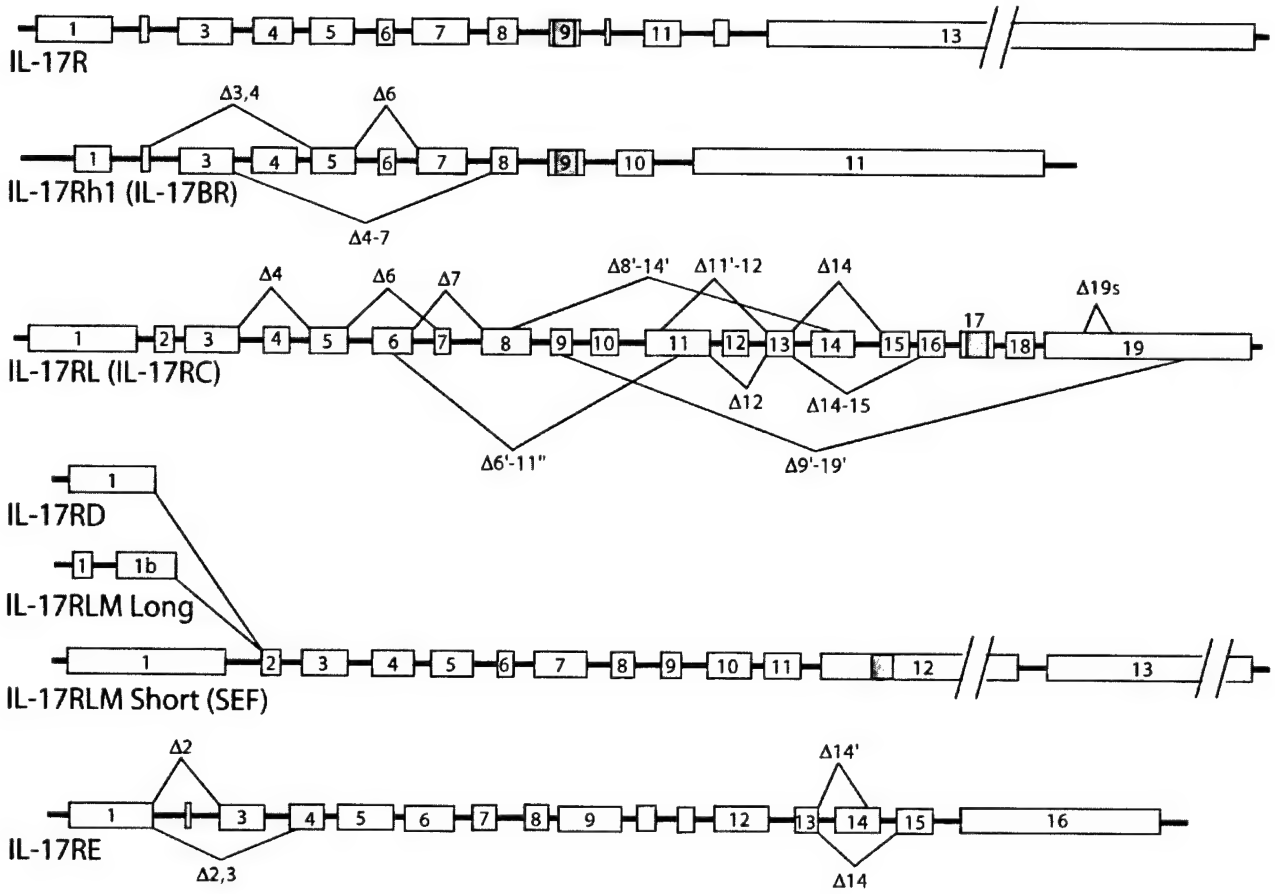

Fig. 2. IL-17 receptor family genomic structure: a schematic representation of the sizes of exons (open boxes) and introns. Shaded areas correspond to the predicted transmembrane domains. Lines connect exons that were joined in sequences from the EST database which represent alternative splicing events. Exons with (′) or (″) have multiple splice donor or acceptor sites evident from sequences in the EST database.

Articular cartilage is a critical component of diarthroidal joints, providing a low-friction surface for articulation. The major components of cartilage matrix include aggrecan, hyaluronic acid, and type II collagen. Aggrecan is a proteoglycan with many negatively charged glycosaminoglycan (GAG) side chains, which functions to retain water and provide resistance to the compressive forces encountered in the joint. Type II collagen provides resistance to tensile forces and helps maintain tissue stability during articulation.

Arthritis is a degenerative disease of articular cartilage causing gradual permanent compromise of joint function. Although the incidence of arthritis increases with advanced age, it can affect people of any age. It already affects more than 42 million Americans in its chronic form, and by the year 2020 the United States Center for Disease Control estimates that it will affect more than 60 million, with 12 million disabled by the disease. Osteoarthritis is a non-inflammatory disease thought to be caused by the "wear and tear" of life, perhaps accelerated by physical damage to the joint. Rheumatoid arthritis is considered an autoimmune disease marked by increased joint inflammation, T-cell infiltration of the synovium, and the involvement of many catabolic cytokines.

Progressive destruction of articular cartilage and bone along with chronic inflammation of the synovium are well documented in rheumatoid arthritis. The infiltration of T-cells into the synovium and the resultant pathology involves a dynamic interaction between the subintimal endothelial cells and the synovium. Activated T-cells secrete detectable amounts of interleukin-17A into the synovial fluid [12]. These increased levels of IL-17A induce a multitude of factors contributing to the degradation of the articular cartilage and erosion of the underlying bone.

3.1. Ex vivo modeling systems help elucidate IL-17s role in joint destruction

Interleukin-17A consistently up-regulates IL-6 [13–20] in both explant cultures and cell cultures of cartilage, synovium, and bone tissues. Interleukin-6, a potent mediator of inflammation in joints, is known to contribute to the overall degradation of cartilage in rheumatoid arthritis. Interleukin-17A has been shown to up-regulate nitric oxide (NO) production and also to increase the mRNA levels of inducible nitric oxide synthase (iNOS) in osteoarthritic cartilage, fetal bone, and meniscus explant cultures, as well as

in cultured osteoblasts and chondrocytes from both normal and osteoarthritic cartilage [13,21–25]. Increased NO levels lead to destruction of the extracellular matrix and chondrocyte damage, contributing to the overall reduction in joint function [26,27].

The enzymatic degradation of cartilage proteoglycans and collagen is mediated through the release of matrix metalloproteinases (MMPs) and plays an important role in arthritis [17,28]. IL-17A has been shown to enhance matrix degradation by inducing the release of cartilage proteoglycan GAGs and collagen fragments, and at the same time inhibit the synthesis of new proteoglycans and collagens [17,18,22,29–31] The anti-inflammatory cytokine IL-4 has been shown to overcome the IL-17A-induced inhibition of proteoglycan synthesis by chondrocytes [26,27].

Interleukin-17A has been shown to synergistically or additively augment many of the destructive effects of IL-1 and tumor necrosis factor alpha (TNF-α) in cartilage, synovium, and meniscus [14,18,19,23]. These cytokines have both been shown to promote arthritic disease, and inhibition of their activity by function-blocking antibodies and soluble receptors or antagonists are currently being evaluated clinically for the treatment of arthritis. While synergy between the IL-17A and IL-1β pathways has been documented, studies in IL-1β knockout mice have shown that IL-17A also promotes arthritis in an IL-1β independent manner [32].

The increased levels of IL-17A in the synovial tissues and fluid of rheumatoid arthritis patients can be a stimulator of osteoclastogenesis through the up-regulation of osteoclast differentiation factor (ODF, osteoprotegerin) [33]. Since osteoclasts function to resorb bone, their increased numbers and prolonged survival may be contributing factors to the bone erosion that is common in arthritis (reviewed in [109]).

The direct catabolic actions of IL-17A on cartilage renders it a potential target in therapeutics for arthritis [18,22,30,32,34]. Studies using a soluble IL-17 receptor have shown that blocking IL-17A activity can inactivate many of its negative effects in animal models of arthritis and in cell culture experiments.

Table 2 shows a survey of the biological activities of IL-17 cytokines in musculoskeletal tissues with references to the primary literature. Fig. 3 is a diagrammatical representation of a chondrocyte highlighting the various matrix components and how they are influenced by the anabolic growth factors and catabolic cytokines. It illustrates the complex relationships between these many factors.

Interleukin-17A has been the primary IL-17 family member studied in arthritis. IL-17F and IL-17E have a similar effect on cartilage proteoglycan release and inhibition of matrix synthesis [6,24,35]. The source of IL-17A and IL-17F are the activated T-cells, and it was unclear whether cartilage itself could produce IL-17 cytokines.

3.2. *Identification of IL-17B in articular cartilage extract*

We hypothesized that there were anabolic factors and inhibitors in articular cartilage that were yet to be identified and used a protein chemistry approach to examine an extract

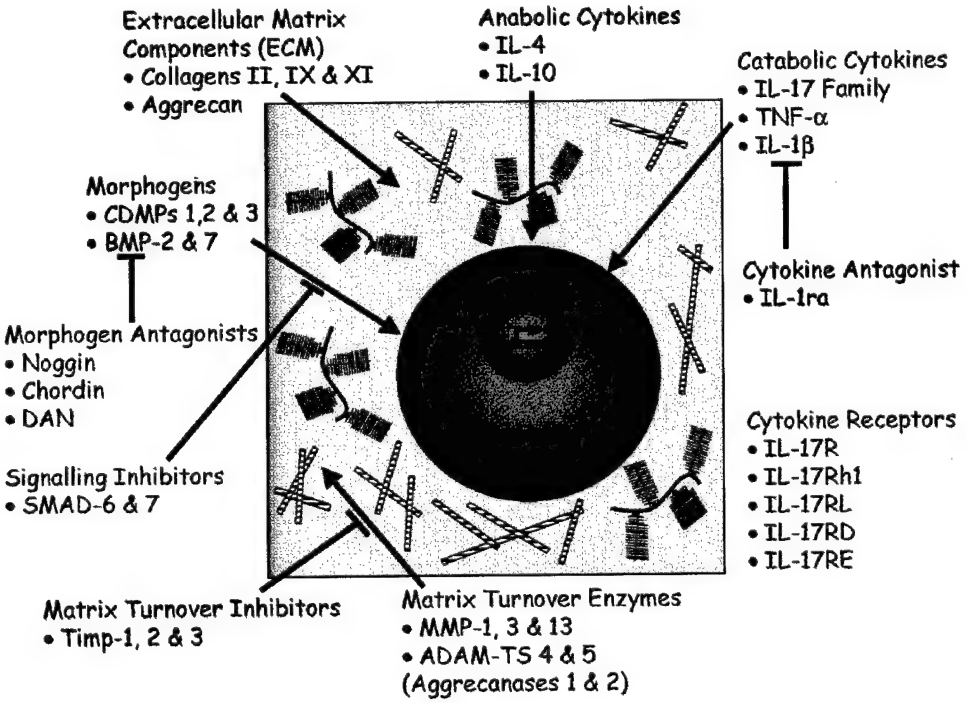

Fig. 3. Cartilage metabolism: a graphical representation of articular cartilage showing the complex relationship between anabolic growth factors and catabolic cytokines involved in extracellular matrix maintenance.

Table 2
Review of IL-17 cytokines in musculoskeletal tissues

Tissue	Model system	Biological effects	Positive interactions	Negative modulation	No modulation	Reference
Cartilage						
Cartilage explants	Articular cartilage explant + IL-17F	↑ Matrix release, IL-6, ↓ proteoglycan synthesis				[6]
	Articular cartilage explant + IL-17A, IL-17F	↑ NO, matrix breakdown, ↓ proteoglycan synthesis	Additive with IL-1α	Inhibited by dexamethasone, anti-LIF		[35,50]
	Articular cartilage explant + IL-17A, IL-17E	↑ Aggrecanase, NO, ↓ proteoglycan synthesis	Additive with IL-1α	Inhibited by anti-LIF, actinonin	No change in IL-2, IL-4, IL-5, IFN-γ mRNA	[24]
	Osteoarthritic articular cartilage explant + IL-17A	↑ NO		Cycloheximide, NF-κB inhibitors	No effect of dexamethasone	[25]
	Nasal cartilage explant + IL-17A	↑ Proteoglycan release				[29]
		↑ Collagen release	Synergism with IL-1α, OSM, TNF-α	Inhibited by IL-4, IL-13, TGF-β1, IGF-1, TIMP-1, BB-94		[29]
Cartilage explants	Patellae explant culture + IL-17A	↓ Proteoglycan synthesis, ↑ proteoglycan release	Synergism with TNF-α			[18]
Mouse arthritis	Collagen II-induced arthritis	↑ IL-17A mRNA	IL-17A augments joint destruction	Blocking IL-17A inhibits arthritis	Independent of IL-1β pathway	[32]
	Collagen II-induced arthritis + IL-4	↓ IL-17A, IL-12, IL6, OPGL, collagen release			No effect on OPG	[26]
	Collagen II-induced arthritis + IL-17A	↑ RANKL, IL-1β, arthritis severity, chondrocyte death			Independent of IL-1α/β pathways	[51]
	Single IL-17A injection into mouse knees	↓ Cartilage proteoglycan content			No leukocyte infiltration or detectable inflammation	[30]
	Multiple IL-17A injections into mouse knees	↓ Cartilage proteoglycan content, ↑ inflammation			No effect on proteoglycan synthesis rate	[30]
Arthritis	Synovial fluid cytokine levels	↑ IL-17, IL-15 in RA but not OA patients	↑ IL-17A mRNA in PBMC by IL-15, IL-2, PMA + ionomycin		LPS, TNF-α, IL-8, IL-6 do not affect IL-17A mRNA in PBMC	[12]
Rat arthritis	Adjuvant-induced arthritis	↑ IL-17A, TNF, IFN-γ, ↓ IL-2, IL-4 in lymph node			No change in TGF-β expression	[52]
	Adjuvant-induced arthritis + soluble IL-17R	↓ Severity of arthritis with increasing amount of soluble IL-17R				[53]
OA cartilage	Passaged chondrocytes + IL-17A	↑ NO, iNOS, activation of MAPKAPK-1,2	Synergism with TNF-α, additive with LIF	PKA, PKC, p38, NF-κB, MEK-1/2 inhibitors	No synergy with IL-1β	[21]
		↑ Activation MEK-1/2, p44/42, MKK-3/6, p38, IκB-α			Change in SAPK/JNK only with PKA inhibitors present	[21]
Chondrocytes	Primary or 1st passage normal chondrocytes + IL-17A	↑ NO, IL-1β, IL-6, iNOS, COX-2, stromelysin	Signals through ERK 1/2, JNK, p38, NF-κB	Inhibited by dexamethasone, p38 inhibitor		[13]
Synovium						
Rheumatoid synovium	Cell culture + soluble IL-17R	↓ IL-6, MIP-3α, C-propeptide of type I collagen	IL-6, MIP-3α additive with soluble IL-1R, TNFR			[22,31]
	Cell culture + IL-17A	↑ MIP-3α mRNA, protein	Synergism with TNF-α	Inhibited by IL-4, IL-13	No synergism with IL-1β, no effect of IL-10	[34]
	Explant culture + IL-17A	↑ IL-6, Col-I degradation, ↓ Col-I synthesis	Synergism with TNF-α	Inhibited by anti-IL-17A		[17,18]

	Explant culture	Explants produce IL-17A, IL-6		IL-4 and IL-13 inhibited production	IL-10 had no effect on IL-17A production	[15]
Synovial fibroblasts	Cell culture + IL-17A	↑ IL-6 mRNA, greater ↑ IL-6 protein	Synergism with TNF-α, sequential effects			[19]
	Cell co-culture with resting T-cells	↑ IL-6, IL-8, PGE2	Synergism with IL-17A			[54]
	Cell co-culture with resting T-cell bank	↑ IL-17A expression correlates to catabolic effect				[55]
	Cell culture + IL-17A	↑ MMP-1	Additive with IL-1β		No effect on TIMP-1 expression	[17]
	Cell culture + IL-17A	↑ IL-6, LIF	Synergism with IL-1β	IL-4 and IL-13 inhibited production of LIF		[14]
	Cell culture + IL-17A	↑ IL-8, Gro-α, Gro-β	IL-17R levels increase with cyclosporin, methotrexate, dexamethasone	Inhibited by blocking p38, PKC, and tyrosine kinases	No effect of pentoxifylline or indomethacine on IL-17R	[56]
	Cell culture + IL-17A	↑ OCIF (= OPG), PGE2		Inhibited by blocking COX-2		[57]
Bone						
Osteoblast	Co-culture of osteoblasts and activated T-cells	↑ IL-6 by osteoblasts		Effect inhibited by CsA	Not inhibited by anti-IL-17A neutralizing antibodies	[16]
	Cell culture + IL-17A	↑ NO, NOS2 only with TNF-α		Inhibited by blocking NF-κB	No synergism with IL-6	[58]
	Co-culture osteoblasts with bone marrow cells + IL-17A	↑ TRAP, PGE2		OPG, anti-IL-17A, inhibitors of COX-2		[33]
	Cell culture + IL-17A with endothelin-1 or PGF2α	↑ IL-6			Downstream of p44/p42 MAP kinase	[20]
Fetal bone	Explant culture + IL-17A and TNF-α	↑ NO, Ca release		Partially inhibited by blocking NF-κB	Insensitive to OPG, NF-κB-independent IL-17A activity?	[22]
	Explant culture + IL-17A and TNF-α	↑ Ca release with IL-17A			No effect of IL-17A and IL-1β on Ca release	[59]
Bone explants (RA)	Bone explants + soluble IL-17R	↓ IL-6, C-propeptide of type I collagen			Soluble IL-17R did not affect IL-6 mRNA in OA synovium	[22]
	Bone explants + IL-17A	↑ IL-6, Col-I degradation, ↓ Col-I synthesis		Partially blocked by soluble IL-17R		[18]
Meniscus						
Osteoarthritic menisci	Meniscus explant culture + IL-17A	↑ NO, ↑ prostaglandin E2 with TNF or IL-1	Synergism with TNF-α and IL-1β			[23]

This represents a survey of the biological activities of the IL-17 cytokine family in related musculoskeletal tissues.

of 2 kg of bovine articular cartilage. Cartilage proteins were extracted in guanidine, fractionated on cation exchange and reverse-phase HPLC columns, then run on 2D SDS-PAGE. One protein identified using this technique was the then-unknown IL-17B, and based on the intensity of the Coomassie-stain we estimate that it is present at a concentration of about 50 ng/g of bovine articular cartilage. The presence of IL-17B mRNA in chondrocytes was confirmed using northern blot and RT-PCR. Fig. 4 shows the expression of IL-17B by immunohistochemistry of chondrocytes in three zones of normal bovine articular cartilage. While the surface chondrocytes show little reactivity, the mid and deep zones are IL-17B positive. The polyclonal antibody to IL-17B shows no cross-reactivity to IL-17A, although it has not been tested against the remaining IL-17 cytokines.

The presence of IL-17B in cartilage and its synthesis by chondrocytes led us to search for the presence of additional IL-17 receptors in cartilage. Immunoblot of cartilage extracts show the presence of both the long and short forms of IL-17RH1. The long form of IL-17RH1 is a transmembrane receptor which has been shown to bind to IL-17B and IL-17E and cause activation of NF-κB [36]. Alternatively spliced variants of this protein are secreted as soluble proteins since they lack the transmembrane domain.

We have identified and cloned a third receptor sharing ~22% identity and 34% similarity with IL-17R and named it

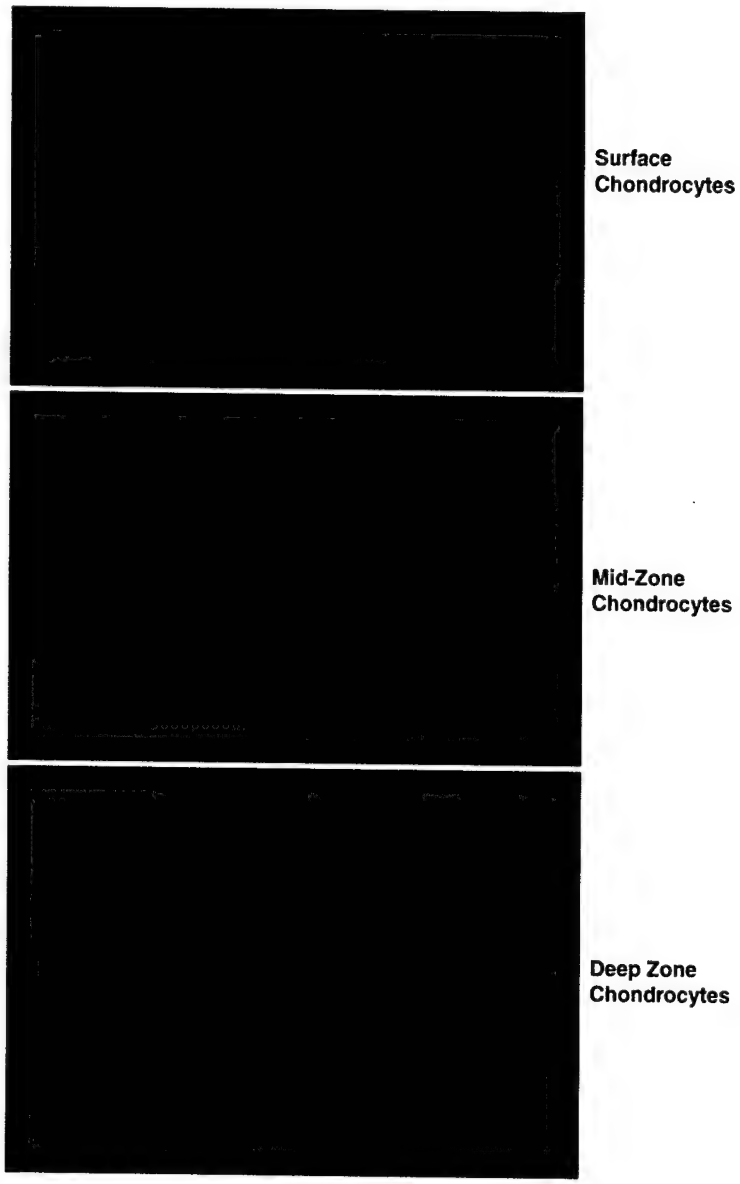

Fig. 4. Chondrocytes in bovine articular cartilage highly express IL-17B: immunohistochemistry of articular chondrocyte cell surface shows IL-17B expression in mid and deep zones but less in surface zone. This figure is a compilation of three separate images taken on a Zeiss LSM 510 confocal microscope. Staining of IL-17B was done using IL-17B specific rabbit antibody (anti-N-terminal-IL-17B) followed by FITC labeled anti-rabbit IgG secondary antibody. Nuclear staining was done by propidium iodide.

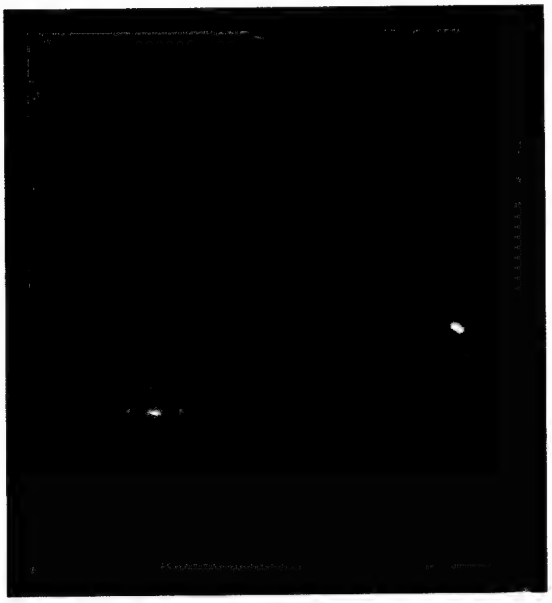

Fig. 5. Interleukin-17 receptor-like molecule (IL-17RL) is expressed in human articular chondrocytes: immunohistochemistry of chondrocytes in mid- and deep-zone articular cartilage show surface expression of IL-17RL. Image was taken on a Zeiss LSM 510 confocal microscope. Staining of IL-17RL was done using IL-17RL specific rabbit antibody (anti-N-terminal-IL-17RL) followed by FITC labeled anti-rabbit IgG secondary antibody. Nuclear staining was done by propidium iodide.

IL-17 receptor-like (IL-17RL) [9]. The cytoplasmic domains of these proteins are even more conserved, sharing 25% identity and 41% similarity across their membrane domains. Fig. 5 shows that IL-17RL is produced by chondrocytes in the mid- and deep-zone human articular cartilage. As with IL-17RH1, alternatively spliced variants of this protein are also secreted since they lack the transmembrane domain. The antibody used for histochemistry in Fig. 5 recognizes the extracellular domain and thus cannot distinguish between soluble and transmembrane isoforms.

During development, cartilage is formed by the actions of anabolic growth factors including bone morphogenetic proteins (BMPs), cartilage derived morphogenetic proteins (CDMPs), and growth and differentiation factors (GDFs). In diseases such as arthritis, cartilage is destroyed through the actions of catabolic cytokines including IL-17, IL-1, and TNF-α. During the homeostasis of healthy tissues, it is likely that there is a balance between anabolic and catabolic factors. The exact composition of factors contributing to this balance may affect a tissue's potential for repair and regeneration. Although bone and articular cartilage are adjacent tissues there is a profound difference in their potential for regeneration and repair; articular cartilage is recalcitrant to repair while bone has immense potential for regeneration.

The differences in innate regeneration potential may be due to concentration of morphogens and associated binding proteins such as noggin chordin and DAN family [37]. For example, in partial thickness defects confined to articular cartilage there is no attempt to initiate repair. However, in full thickness defects, when the subchondral bone is penetrated, there is initiation of repair of articular cartilage implying a role for subchondral bone. The bone matrix is a repository of bone morphogenetic involved in cartilage and bone morphogenesis. Thus, the difference between bone and articular cartilage may be due endogenous growth factors and morphogenetic proteins and associated binding proteins and catabolic cytokines. Other factors that may influence the lack of repair of damaged cartilage are that cartilage is a tissue comprised of immobile cells fixed in a tightly cross-linked extracellular matrix. Also, unlike in bone, in cartilage there is no population of mesenchymal progenitor cells. The initiation of cartilage morphogenesis is governed by BMPs. The newly formed articular cartilage is maintained by insulin-like growth factor-1 (IGF-1) and platelet-derived growth factors (PDGFs). The homeostasis of articular cartilage is the function and balance of anabolic morphogenetic proteins, and catabolic cytokines such as interleukin-1 (IL-1), interleukin-17 (IL-17), tumor necrosis factor alpha (TNF-α). Therefore at steady state the articular cartilage is maintained by an interplay between cartilage morphogens, cognate antagonists and catabolic cytokines (Fig. 3).

4. IL-17 in cancers

The mis-regulation of growth factor pathways is a common feature of many cancers. Although there are no published reports describing genetic linkage of either IL-17 cytokines or receptors directly to cancers, there is evidence that IL-17s are active in cancers. IL-17A has been shown to promote angiogenesis in tumor models and correlates well with the numbers of blood vessels in human ovarian cancers [38]. IL-17A promotes tumorgenicity of human cervical tumors in nude mice and is associated with an increased level of IL-6 expression at the tumor sites [39]. Increased levels of IL-6 correlate well with the invasiveness of cervical tumors [40]. These reports indicate a role of IL-17 cytokines in promoting tumor. However, other lines of evidence indicate that IL-17A may protect against tumors by promoting immune system-mediated tumor rejection [41–43]. Table 3 is a survey of the biological activities of IL-17s in cancers.

4.1. IL-17 in prostate cancer

Prostate cancers generally metastasize to bones such as the spine and the pelvis. Prostate metastases lead to both osteoblastic and osteolytic lesions in bone. The dynamic regulatory networks at the interface of prostate carcinoma metastases and bone are indicated in a simplified form in Fig. 6. The interactions between stromal cells and epithelial cells are critical for tumor progression and metastasis in prostate. The carcinoma cells secrete morphogens and growth factors such as BMPs, IGF and TGF-β which act on

Table 3
Review of IL-17 cytokines in cancer

Tissue	Model system	Biological effects	Positive interactions	Negative modulation	No modulation	Reference
Prostate carcinoma	Biopsy	Altered IL-17RL distribution in grades of cancer				[9]
Fibrosarcoma	IL-17A overexpression, rejection model	↑ Rejection of IL-17A expressing tumor cells	T-cell dependent	Anti-CD4, CD8, CD90	Anti-asialo GM1	[41]
CHO cells	IL-17A overexpression, nude mice	↑ Matrigel invasion, lung mets, NK activity			No effect on proliferation, sq tumor growth	[42]
	Cell culture + IL-17B				No effect on IL-6, IL-8, TNF-α, IFN-γ, IL-3, G-CSF	[7]
Leukemic monocyte	THP-1 cell culture + IL-17B	↑ TNF-α, IL-1β			No effect on IL-6, IL-1α, IFN-γ, G-CSF	[60]
	THP-1 cell culture + IL-17C	↑ TNF-α, IL-1β			No effect on IL-6, IL-1α, IFN-γ, G-CSF	[60]
Murine leukemia	Viral integration site analysis	↑ Evi27 in murine myeloid leukemias				[8]
Cervical tumors	Cervical tumor cells + IL-17A	↑ IL-6, IL-8 mRNA, protein			No effect on in vitro proliferation	[39,40]
	IL-17A overexpression, nude mice	↑ Tumor size, macrophage recruitment, IL-6				[39]
Hematopoietic	IL-17A overexpression, mice	↓ Tumor size in immunocompetent mice			No effect on tumor size in nude mice	[43]
Ovarian cancer	Ovarian cancer biopsy	+ Correlation between IL-17A and angiogenesis			No correlation to tumor stage, survival	[38]

This represents a survey of the biological activities of the IL-17 cytokine family in cancer.

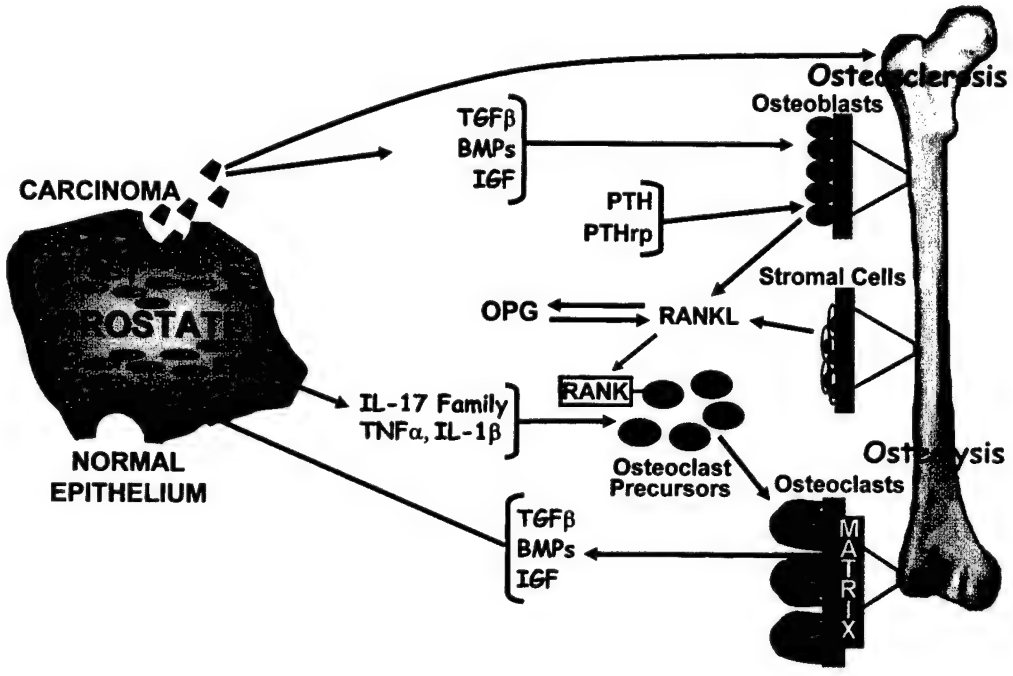

Fig. 6. Cancer metastasis to bone: a graphical representation of the authors views of how the progression of a metastatic tissue such as prostate cancer can lead to the progression of osteosclerosis as well as osteolysis.

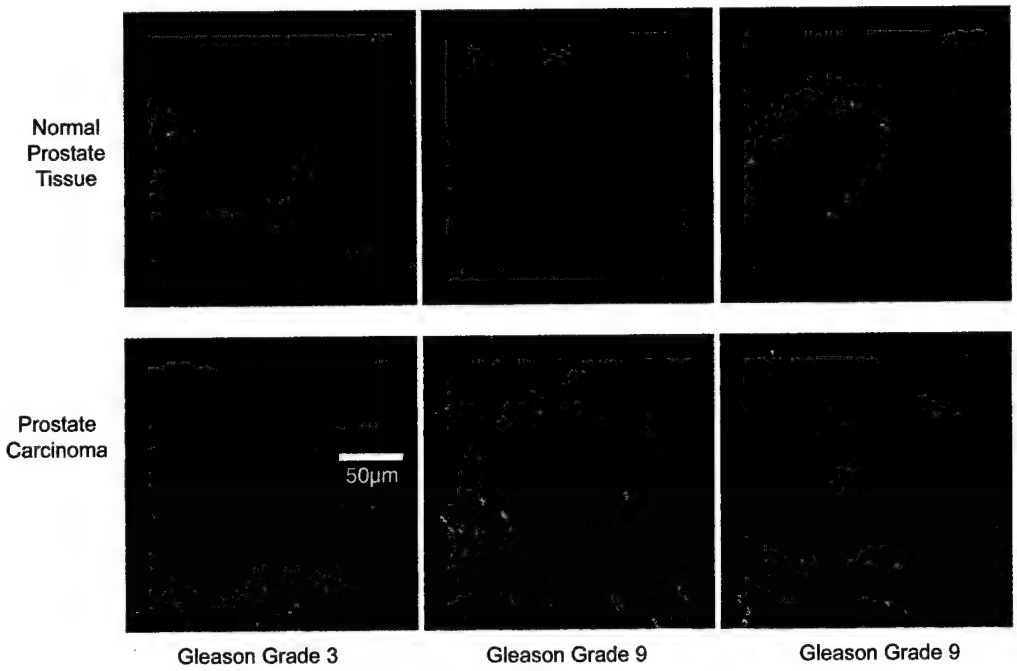

Fig. 7. Interleukin-17 receptor-like molecule (IL-17RL) is expressed in human prostate: immunohistochemistry of normal prostate and increasing Gleason grades of prostate cancer show surface expression of IL-17RL. The cancerous tissues show some evidence of a shift from epithelial expression to stromal expression as the cancer becomes more severe. Image was taken on a Zeiss LSM 510 confocal microscope. Staining of IL-17RL was done using IL-17RL specific rabbit antibody (anti-N-terminal-IL-17RL) followed by FITC labeled anti-rabbit IgG secondary antibody. Nuclear staining was done by propidium iodide (image used with permission of the author, D.R. Haudenschild and publisher).

cells in the bone. In response to PTH and PTHrp osteoblasts and stromal cells secrete RANK ligand (RANKL) which binds to receptor activator of NF-κB (RANK) on osteoclast precursors to differentiate into functional multinucleate osteoclasts. The bioavailability of RANKL to its receptor RANK is determined by the activity and affinity of a soluble decoy receptor osteoprotegerin (OPG) to RANKL. Interleukin-1, interleukin-17, tumor necrosis

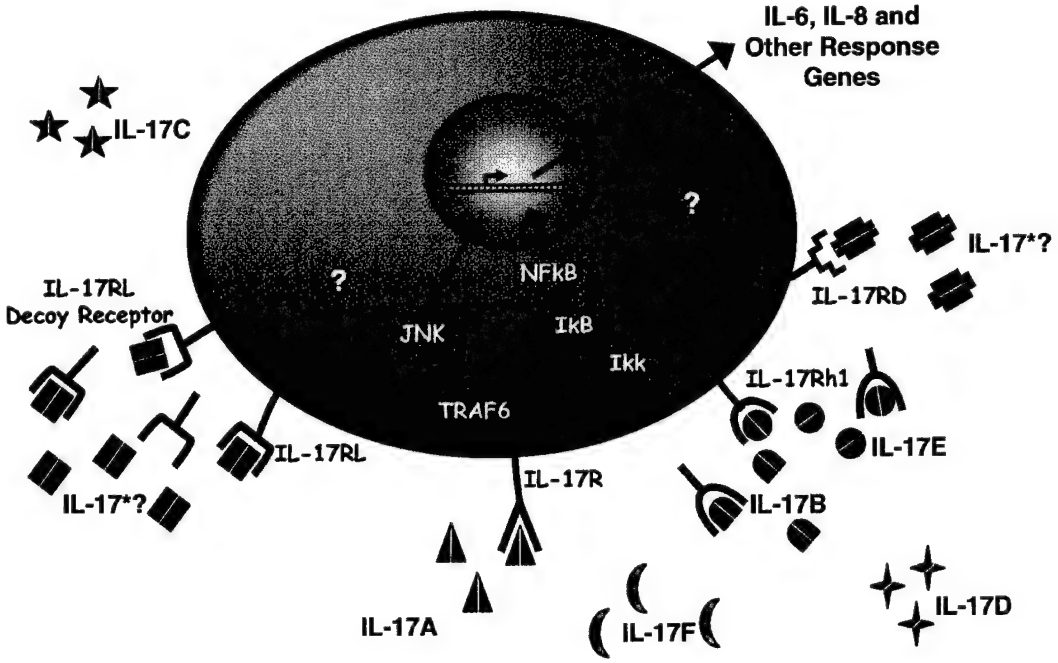

Fig. 8. Cellular signaling of IL-17 cytokines: a graphical representation of the known IL-17 cytokines and ligands. The transmembrane receptors as well as their soluble decoy receptor versions are shown. Some of the signal transduction pathways are represented with question mark in the place of unknown pathways. There are no known receptors for IL-17C, IL-17D and IL-17F. There are no known ligands for IL-17RL, IL-17RD or IL-17RE.

Table 4
Review of IL-17 cytokine signal transduction

Experimental system	Cytokine used	Signal transduction through these actions	Inhibitors of signal transduction	Not involved in signaling	Other effects	Reference
Subepithelial myofibroblasts	SEMF cell culture + IL-17A	NF-κB, p42/44 ERK 1/2, p38	Inhibitors of MAPK		↑ IL-6 mRNA stability	[85]
Embryonic fibroblasts	Cell culture + IL-17A, TRAF-6 KO	TRAF-6, IKK, JNK, NF-κB, AP-1		TRAF-2	TRAF-6 directly binds IL-17R	[103]
Monocytic leukemia	Cell culture + IL-17A	JAK 1,2,3, Tyk 2, STAT 1,2,3,4				[44]
NIH-3T3 cells		↑ Stability of G-CSF mRNA by ↓ degradation		JAK/STAT	↑ G-CSF mRNA stability	[104]
Peripheral blood leukocyte		Identification of IL-17A receptor			Affinity lower than biological activity	[105]
Pancreatic myofibroblasts	Cell culture + IL-17A	MEK-1/2 and p38	Inhibitors of MEK-1/2 and p38		↑ IL-6 mRNA stability	[106]
Glioblastoma	Cell culture + IL-17A	↑ IκB-α mRNA, protein degradation				[75]
Intestinal epithelium	Cell culture + IL-17A	↑ NF-κB activation, p65/p50 subunits, TRAF-6		TRAF-2 not required		[80]
Macrophage	Cell culture + IL-17A	NF-κB, AP-1, CREB, and ↑ calcium flux	Inhibitors of PKC, MAPK			[107]
Vascular epithelium	Cell culture + IL-17D	NF-κB activation				[65]
	Cell culture + IL-17F (ML-1)	ERK 1/2		No activation of p38 or JNK		[72]
OA cartilage	Explant culture + IL-17A	NF-κB activation				[25]
OA cartilage	Passaged chondrocytes + IL-17A	MEK-1/2, p44/42, MKK-3/6, p38, IκB-α, MAPKAPK-1,2	Inhibitors of PKA, PKC, p38, NF-κB, MEK-1/2	Activation of SAPK/JNK only with PKA inhibitors present		[21]
Chondrocytes	Cell culture + IL-17A	ERK 1/2, JNK, p38, NF-κB	Inhibitors of p38, IKK			[13,108]

This represents a survey of the signal transduction pathways of the IL-17 cytokine family in a variety of tissues examined.

Table 5
Review of biological activities of IL-17 cytokines in the immune system

Tissue	Model system	Biological effects	Positive interactions	Negative modulation	No modulation	Reference
Immunology						
Dendritic cell progenitor	Bone marrow cells + IL-17A and GM-CSF	↑ CD11c, CD40, CD80, CD86, MCH class II				[61]
Neutrophil migration	IP injection of IL-17B, neutrophil count	↑ Neutrophil influx				[7]
Viral IL-17	Mutant Herpesvirus saimiri (no IL-17) infecting T-cells				No effect on viral replication or transformation of cells	[62]
T-cell proliferation	Cell culture + IL-17A or vIL-17	↑ Proliferation				[63]
Macrophage	Cell culture + IL-17A	↑ MMP-9, PGE2, COX-2, STAT 1,3 phosphorylation, AP-1 binding		COX inhibitor, IL-4, IL-10, IL-13, inhibitors of p38, MEK-1/2, NF-κB	No effect on MMP-1, MMP-3, JNK/SAPK phosphorylation	[28]
	Cell culture + IL-17A	↑ TNF-α		Blocked by PGE2 through Egr-1		[64]
Hematopoiesis						
Bone marrow cells	Colony formation in methylcellulose + IL-17D	↓ Myeloid progenitor cell proliferation				[65]
	Colony formation of marrow cells + IL-17F				No effect of IL-17F on colony formation	[4]
	Cell culture + IL-17A	↑ IL-6 protein				[66]
Transplantation						
Vascular	Thoracic aorta transplants + soluble IL-17R:Fc	↓ MNC infiltration early			No effect of IL-17R:Fc on chronic rejection	[67]
Kidney	Renal transplants, rejection	↑ IL-17A in renal transplant rejections				[68–70]
Heart	Heart transplant + soluble IL-17R:Fc	↑ Cardiac allograft median survival time				[71]
		↓ T-cell proliferation, ↑ allograft survival				[61]
Vasculature						
Vascular endothelium	Cell culture + IL-17D	↑ IL-6, IL-8, GM-CSF	Signals through NF-κB activation		IL-1β, IL-2, IL-4, IL-5, IL-10, IL-12, IFN-γ, TNF-α	[65]
Vascular endothelium	Capillary tubule formation + IL-17F	↓ Tubule formation				[4]
	HVEC + IL-17F	↑ TGF-β1, TGF-β2, MCP-1, Lymphotoxin-β, IL-2				[4]
	HUVEC + IL-17F (ML-1)	↑ IL-6, IL-8	Signals through ERK 1/2		No activation of p38 or JNK	[72]

Table 6
Review of biological activities of IL-17 cytokines in other tissues

Tissue	Model system	Biological effects	Positive interactions	Negative modulation	No modulation	Reference
Brain						
	Ischemia animal model	↑ IL-17A mRNA locally after ischemia				[73]
		↑ IL-17A mRNA systemically				[73]
Neurons	In situ hybridization/antibody stains	IL-17B expressed by motor and sensory neurons				[74]
Glioblastoma	Rat cell culture + IL-17A	↑ IκB-α mRNA, IκB-α protein degradation				[75]
		↑ IL-6, IL-8 mRNA	Synergism with IL-1β			[75]
Skin						
	Keratinocyte cell culture + IL-17A	↑ IL-6 mRNA	Synergism with IFN-γ		IL-1α, IL-15, GAPDH, proliferation	[76]
	Keratinocyte cell culture + IL-17A	↑ IL-8 mRNA	Synergism with TNF-α, IFN-γ		ICAM-1, HLA-DR, MHC class I, CD40	[76,77]
	Keratinocyte cell culture + IFN-γ	↑ ICAM-1, MHC class I, CD40, RANTES, IL-1α	↑ ICAM-1 synergy with IL-17A	↑ Of RANTES blocked by IL-17A	MHC class I, CD40	[76,77]
	Keratinocyte cell culture + TNF-α	↑ ICAM-1, RANTES		↑ Of RANTES blocked by IL-17A	ICAM-1	[77]
	Keratinocyte cell culture + IL-17A	↑ CCL-20 mRNA (= MIP-3α)	Synergism with TNF-α			[78]
	Foreskin fibroblast + IL-17F	↑ IL-8, G-CSF protein				[6]
	Foreskin fibroblasts + IL-17B				No effect on IL-6	[60]
	Foreskin fibroblasts + IL-17C				No effect on IL-6	[60]
	Foreskin fibroblasts + IL-17A	↑ IL-6, IL-8, ICAM-1				[79]
Digestive tract						
Intestinal epithelium	Intestinal epithelia cells + IL-17A	↑ NF-κB activation, p65/p50 subunits				[80]
		↑ CINC	Synergism with IL-1β, TRAF-6 dependent		TRAF-2 not required	[80]
		↑ IL-8 mRNA, protein				[80]
		↑ MCP-1				[80,81]
Intestinal epithelium	*Helicobacter pylori* infection	↑ IL-17A when infected				[82]
		↑ IL-8 mRNA, protein				[82]
Intestinal epithelium	Fetal cell cultue + IL-17A	↑ IL-8 mRNA, protein	Synergism with IFN-γ		IFN-γ alone does not affect IL-8	[81]
		↑ MCP-1 mRNA, protein	Synergism with IFN-γ			[81]
Pancreas	Pancreatic myofibroblast cells + IL-17A	↑ IL-6 mRNA, protein	Synergism with TNF-α, IFN-γ			[83]
		↑ Stability of TNF-α-induced IL-6 mRNA			IFN-γ-induced IL-6 mRNA not stabilized	[83]

Colon carcinoma	T84 cell culture with IL-17A	↑ Tight junction formation ↑ Claudin-1,2 mRNA, protein			IL-17A	[84] [84]
Rat jejunitis	Indomethacin injection, small intestine examined	↑ IL-17BR				[7]
Subepithelial myofibroblasts	SEMF cell culture + IL-17A	↑ IL-6, IL-8, MCP-1 protein	Synergism with TNF-α and IL-1β, signals through NF-κB, p38, p42/44 ERK	Inhibited by MAPK inhibitors		[85]
		↑ Stability of TNF-α-induced IL-6 mRNA		Inhibited by p38 inhibitor		[85]
Lung						
	Asthma	↑ IL-17A in lungs of asthma patients				[86]
	Primary bronchial epithelial cells + IL-17A	↑ IL-8, Gro-α, G-CSF mRNA, protein	Synergism with TNF-α		Dexamethasone on IL-8 production	[86]
		↑ IL-6				[86]
	Primary bronchial epithelial cells + IL-17A	↑ IL-6, IL-8 protein	Synergism with IFN-γ	PD98059 (MEK-1/2 inhibitor)		[87]
		No effect on RANTES, ICAM expression	IL-17A augments IFN-γ's effect on ICAM			[87]
	Primary bronchial epithelial cells + IL-17F (ML-1)	↑ IL-6, IL-8 ICAM-1 mRNA, protein			No effect on eotaxin, RANTES mRNA, c-JUN or JNK activation	[72,88]
	Intratracheal instillation of IL-17A	↑ Neutrophil recruitment		Dexamethasone inhibits neutrophil recruitment	No recruitment of eosinophils, macrophages, lymphocytes	[89,90]
	Bronchoalveolar lavage specimens	↑ IL-17A in eosinophils of asthma patients				[91]
	Bronchial fibroblast culture + IL-17A	↑ IL-6, Gro-α mRNA in normal and asthma				[91]
		↑ IL-11 mRNA only in asthmatic patients		Dexamethasone inhibits IL-17A-induced IL-6, IL-11	No effect of IL-17A on IL-11 in non-asthma	[91]
	Serum IL-17A levels in asthma patients	No significant elevation of IL-17A				[92]
	Klebsiella pneumoniae challenged lung	↑ IL-17A in lungs after bacterial challenge		Alcohol inhibits ↑ IL-17A after bacterial challenge		[93,94]
	K. pneumoniae challenge, IL-17R KO	↑ Mortality in challenged IL-17R KO mice		↓ MIP-1α-1β,-2, G-CSF, SCF in challenged KO mice		[95]
	IL-17A overexpression in lung, adenoviral	↑ TNF-α, IL-1β, MIP-2, G-CSF				[93]
	Rat neutrophil culture + IL-17A				No effect on myeloperixidase activity	[96]
	Bronchial epithelial cells + IL-17A	↑ IL-8 mRNA, protein	Synergism with TNF-α	↑ Of IL-8 blocked by hydrocortisone	No effect of IL-17A on neutrophil migration	[90]
	Severe lung inflammation in humans	↑ IL-17A, IL-6, IL-8, TNF-α				[97]
	Severe lung inflammation in mice	↑ IL-17A, IL-1β, TNF-α, IL-6, MIP, MCP, etc.			No effect on IL-4, IL-5, IL-18 mRNA	[98]

Table 6 (*Continued*)

Tissue	Model system	Biological effects	Positive interactions	Negative modulation	No modulation	Reference
Kidney						
Nephrotic disease	Urine samples of MCNS, IgAN, healthy patients	↑ IL-17A excretion in nephrotic patient			No age or gender-related differences	[99]
Renal epithelia	Primary tubular epithelial cells + IL-17A	↑ IL-6, IL-8, MCP-1, RANTES*	Synergism with CD40L	Inhibited by blocking NF-κB	No effect of IL-17A alone on RANTES	[69,70,100]
Renal biopsy	Segmental glomerulosclerosis				No IL-17A mRNA detected in any biopsy	[101]
Renal carcinoma	293, TK-10 cell culture + IL-17E	↑ NF-κB-responsive luciferase activity, IL-8 protein				[36]
Miscellaneous						
Mouse IL-17E transgenic mouse	Myosin L-chain 2 promotor	Smaller, jaundiced, ↑ cytokine expression				[102]
		Neutrophilia, eosinophilia, multiorgan inflammation				[102]
NIH-3T3 cells	Cell culture + IL-17A	↑ IL-6 protein	Signals through NF-κB activation			[63]

factor (TNF) and their cognate signaling systems have a role in osteoclastogenesis. The degradation of bone matrix by multinucleate osteoclasts releases growth factors and morphogens from the extracellular matrix. The regulatory networks in the breast/prostate cancer metastasis to bone are reciprocal and dynamic as illustrated by the secretion of BMPs, IGF and TGF-β family of ligands, cognate receptors and antagonists and binding proteins for the growth factors and morphogens. The binding proteins include IGF-binding proteins, latent TGF-β binding proteins and BMP antagonists Noggin, Chordin, Gremlin, Cereberus and DAN. The interleukin-17 family of cytokines may thus play a role in bone resorption and lead up to osteolytic fractures.

IL-17A is expressed only in T-cells. We therefore searched for the expression of other IL-17 cytokines in normal and cancerous prostate to gain insight into their possible roles in this tissue. Current versions of the EST database indicate that IL-17B, IL-17C and IL-17E cytokines are expressed in the prostate. We have shown that IL-17RL is expressed in human prostate by immunohistochemistry and RT-PCR [9]. It is noteworthy that in prostate carcinoma the immunoreactivity to extracellular domain shifted to the stroma with advancing Gleason grades, and that there is a progressive loss of staining in the epithelium (Fig. 7). We have quantitative RT-PCR (TaqMan) evidence that exon usage is tissue specific which implies that there are regulatory factors that control the RNA splicing of IL-17RL.

The presence of soluble IL-17RH1 and IL-17RL decoy receptors, and the tissue-specific regulation of IL-17RL mRNA splicing to generate different receptor isoforms, hint that the regulation of IL-17 pathways is complex and tightly regulated.

5. IL-17 signaling pathways

The emerging knowledge about the IL-17 family and IL-17 receptors has set the stage for investigation of signaling pathways. IL-17 Receptor (IL-17R) activates extracellular signal-regulated protein kinase (ERK), c-jun N-terminal kinase (JNK) and p38 MAP kinase pathways [13,21,44,45]. These signaling pathways result in up-regulation of IL-6, IL-1 and NF-κB [46]. The current status of the signaling pathways is presented diagrammatically in Fig. 8, and in Table 4. The emerging novel receptors include IL-17RL (also designated IL-17RC), IL-17RD and IL-17RE. The fact that IL-17 family of ligands unexpectedly revealed a cystine knot similar to the BMP/TGF-β, PDGF and NGF indicate the potential for cross-talk with other morphogen signaling pathways. The potential for IL-17RL and IL-17RH1 to exist as both soluble decoy receptors and signaling transmembrane receptors presents an additional level of control. The soluble decoy receptors may bind to the IL-17 family of ligands selectively and reduce or eliminate their bioavailability.

6. IL-17 biological activity in other tissues

Interleukin-17 cytokines have been studied in a variety of other tissues and diseases. A large body of evidence shows that IL-17A and IL-17F (ML-1) are involved in asthma. Asthma is marked by the recruitment of neutrophilic leukocytes into the airway, a process thought to be regulated by T-cells through pro-inflammatory cytokines such as IL-6 and TNF-α. IL-17A and IL-17F expression are increased in asthmatic versus normal patients, and both cytokines have been shown to induce IL-6 and IL-8 expression [7,29,34,35,103]. This topic is nicely reviewed in [47–49]. Tables 5 and 6 present a survey of the biological activities in the immune system and various other tissues not discussed individually within this text.

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